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Investigating the association of rs2910164 with cancer predisposition in an Irish cohort

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Abstract

Introduction: MicroRNAs (miRNAs) are small noncoding RNA molecules that exert post-transcriptional effects on gene expression by binding with cis-regulatory regions in target messenger RNA (mRNA). Polymorphisms in genes encoding miRNAs or in miRNA–mRNA binding sites confer deleterious epigenetic effects on cancer risk. miR-146a has a role in inflammation and may have a role as a tumour suppressor. The polymorphism rs2910164 in the *MIR146A* gene encoding pre-miR-146a has been implicated in several inflammatory pathologies, including cancers of the breast and thyroid, although evidence for the associations has been conflicting in different populations. We aimed to further investigate the association of this variant with these two cancers in an Irish cohort.

Methods: The study group comprised patients with breast cancer (BC), patients with differentiated thyroid cancer (DTC) and unaffected controls. Germline DNA was extracted from blood or from saliva collected using the DNA Genotek Oragene 575 collection kit, using crystallisation precipitation, and genotyped using TaqMan-based PCR. Data were analysed using SPSS, v22.

Results: The total study group included 1516 participants. This comprised 1386 Irish participants; 724 unaffected individuals (controls), 523 patients with breast cancer (BC), 136 patients with differentiated thyroid cancer (DTC) and three patients with dual primary breast and thyroid cancer. An additional cohort of 130 patients with DTC from the South of France was also genotyped for the variant. The variant was detected with a minor allele frequency (MAF) of 0.19 in controls, 0.22 in BC and 0.27 and 0.26 in DTC cases from Ireland and France, respectively. The variant was not significantly associated with BC (per allele odds ratio = 1.20 (0.98–1.46), $P = 0.07$), but was associated with DTC in Irish patients (per allele OR = 1.59 (1.18–2.14), $P = 0.002$).

Conclusion: The rs2910164 variant in *MIR146A* is significantly associated with DTC, but is not significantly associated with BC in this cohort.

Key Words
- thyroid
- endocrine cancers

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Background

The association between breast and thyroid disorders has been widely explored with a large amount of epidemiological evidence linking breast and thyroid malignancies. However, the extent and explanation for this association have remained ill-defined. A recent meta-analysis has revisited the possibility of such an association and has confirmed the existence of, and quantified the increased co-occurrence of breast and differentiated thyroid cancer (DTC) (1). Both cancers occur predominantly in females, and there is a significantly increased risk of developing thyroid cancer as a second primary malignancy following a diagnosis of breast cancer (BC) and vice versa (2, 3, 4, 5). Mutations in *PTEN* have long been known to predispose to both types of malignancy as part of the *PTEN* hamartoma tumour syndrome (6). A relationship between BC and benign thyroid disease has also been hypothesised (7), given the common iodine transport mechanism (8, 9), prevalence of anti-thyroid peroxidase (TPO) autoantibodies in BC patients (10, 11, 12) and the role of thyroid hormone receptor B in BC (13). A non-syndromic monogenic disorder predisposing to breast and thyroid cancers has been postulated but has not, as yet, been identified (14). However, it is possible that this association may be explained by overlapping moderate- or low-penetrance breast-thyroid cancer genetic susceptibility loci (15).

MicroRNAs (miRNAs) are crucial elements in the regulation of gene expression and are involved in a host of physiological and pathological processes. A substantial proportion of the human transcriptome is subject to regulation by miRNAs (16). MicroRNA genes are transcribed from endogenous DNA into primary miRNA transcripts (pri-miRNA), which are then processed by Drosha-containing complexes to form hairpin structures called pre-miRNAs. Pre-miRNAs are then transported into the cytoplasm and processed further by a Dicer-containing complex, which acts to excise the hairpin loop. Binding of miRNA to target messenger RNA (mRNA) leads to translational suppression or mRNA degradation (17). Partial complementarity is often sufficient for binding (16), meaning that individual miRNAs may have hundreds of different mRNA targets, and the individual mRNA target can be regulated by many different miRNAs leading to a rich and complex miRNA–mRNA network. The potential complexity of the miRNA–mRNA network can be exemplified by the miR-146 family of microRNAs. This family includes two closely related but genetically distinct microRNAs, miR-146a and miR-146b, differing only at two nucleotides in the 3′ region of the mature sequences (17).

These miRNAs are critical in a number of immune and inflammatory response pathways and are activated differentially by NF-KB and in response to pro-inflammatory cytokines (17). miR-146a has a number of molecular targets involved in innate and adaptive immune responses; cell proliferation, invasion and metastasis; including, among others, TRAF6 (17), IRAK1 (17), IRAK2 (18), EGF-R (19), NOTCH1 (20) and ROCK1 (21). miR-146a upregulation is mediated by BRAF and NRAS oncoproteins (20).

The typical human genome varies from the reference sequence at 4.1–8 million sites (22), and the majority of this variation is attributable to small indels and single nucleotide polymorphisms (SNPs). The vast majority of these SNPs are benign, but they may become relevant functionally and clinically if they occur in a critical binding site or regulatory region (23). A single nucleotide variant (rs2910164: G>C) in the precursor stem region of *pre-miR-146a* is thought to reduce the stability of the pri-miR and affect processing of pri- to pre-miRNA, thus impacting expression of mature miR-146a (24). This variant has been implicated in a host of malignant and non-malignant inflammatory conditions such as hepatocellular (25) and gastric cancers (26), coronary artery disease (27), inflammatory bowel disease (28) and multiple sclerosis (29, 30). Some authors report an association between the variant in heterozygous (GC), but not homozygous (CC) states and an increased risk of papillary thyroid cancer compared to wild-type genotype (GG) (24), while others report an association with both heterozygous and homozygous states (31). Data with respect to this association are conflicting, with other groups failing to show an association with hetero- or homozygous genotypes (32, 33). Similarly, there are conflicting reports of the impact of the variant allele on mature miR-146a expression, with some authors reporting reduced expression (24), and others overexpression (34).

A possible association between the rs2910164 variant and BC was suggested after a report by Shen and coworkers suggested an impact of age of onset of familial BC on Chinese patients (34). An Italian study of a small cohort of carriers with *BRCA1/BRCA2* mutations also suggested an influence of age of onset of disease, but not on risk overall (35). However, no association between age at diagnosis or disease risk and genotype was identified in a larger study of *BRCA1/BRCA2* mutation carriers from Europe and USA

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(36) or in a different Chinese cohort of sporadic cases (37). It has been postulated that ethnicity may significantly modify the association between miRNA polymorphisms and cancer risk (38). An association between genotype and sporadic BC risk and phenotype has been investigated in variably powered studies from China (37), Italy (35, 39), Germany (39), Spain (40), Australia (41), Saudi Arabia (42), India (43) and Iran (44, 45, 46); and in numerous meta-analyses with conflicting results (26, 47, 48, 49). To date, no Irish samples have been included in such analyses.

The aims of this study were to investigate the association between the variant allele of rs2910164 in *MIR146A* and predisposition to breast and differentiated thyroid cancers in an Irish patient sample and to investigate the frequency of the variant in a distinct patient population from the South of France.

Methods

Study samples

Case–control analyses were undertaken, comparing genotypic and allelic frequencies of the variant in patients with BC and in those with DTC, to frequencies in unaffected controls.

Unselected patients with confirmed *in situ* or invasive BC ($n=534$) were recruited via a symptomatic and screening BC tertiary referral centre (Galway University Hospital). Of these, 7 were found to carry a pathogenic mutation in *BRCA1* or *BRCA2* and were excluded from analysis. Two other individuals were found to carry pathogenic mutations in another BC susceptibility gene (*CHEK2*, *CDH1*) and were also excluded.

Patients were recruited from thyroid cancer treatment clinics at tertiary centres in the West of Ireland and South of France as part of a collaborative multicentre study to establish a thyroid cancer biobank at the Discipline of Surgery in the Lambe Institute for Translational Research, based in Galway University Hospital. Patients with a histological confirmation of DTC were included ($n=269$). Exclusion criteria included benign thyroid disease, medullary thyroid cancer, or known pathogenic germline mutations in cancer predisposition genes.

Individuals were included as controls if they did not have a current or previous diagnosis of cancer (not including non-melanomatous skin cancer), if they did not have a first-degree familial history of breast, ovarian or thyroid cancers and if they were aged over sixty years. Controls were recruited from non-oncological outpatient clinics, and from volunteers in the community.

Written and informed consent was obtained from each patient, and the study was approved by the Institutional Ethics Review Board at Galway University Hospital.

Data pertaining to tumour clinico-pathological characteristics and previous germline genetic testing were recorded from hospital histopathology and genetic testing reports. Information regarding personal and familial medical history was self-reported by patients.

DNA extraction

Participants recruited in hospital were asked to provide a 10 mL whole blood sample, and those recruited from the community were asked to provide a salivary sample collected using the DNA Genotek Oragene 575 collection kit. DNA was extracted manually by ethanol precipitation from whole blood or saliva, and qualified and quantified using nanodrop spectrophotometry. DNA was deemed to be of suitable purity if the ratio of the absorbance at 260 and 280 nm measured approximately 1.8 (50).

Genotyping

Genotyping was performed by PCR using a TaqMan assay (Applied Biosystems) containing allele-specific probes and a PCR primer pair to detect the specific variant under investigation. Each allelic discrimination reaction mix contained 1 µL TaqMan SNP genotyping assay, 10 µL TaqMan SNP genotyping Master Mix and 40 ng/9 µL genomic DNA. RT-PCR was performed using ABI 7900 HT Fast Real-Time PCR System (Applied Biosystems) under the following conditions: 95°C for 10 min to facilitate activation of DNA polymerase, and 40 cycles of amplification, with denaturation at 95°C for 15 s followed thereafter by annealing and extension at 60°C for one minute. The assay for rs2910164 SNP was manufactured such that reporter dyes were tagged to the 5′ end of alternative allele probes (VIC to the variant C allele probe, FAM to the G allele probe (Thermo Fisher Scientific; www.thermofisher.com/order/genome-database/browse/genotyping/keyword/rs2910164)) and a non-fluorescent quencher at the 3′ end of the respective probes). VIC dye fluorescence only was interpreted as homozygosity for the C allele, FAM dye fluorescence only as homozygosity for the G allele and fluorescence signals from both dyes as heterozygosity. Allelic discrimination plots were generated, with automatically interpreted FAM and VIC fluorescent signals plotted on *X* and *Y* axes. Automated

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genotype calling was confirmed manually by interrogation of multicomponent plots.

Statistical analysis

Data were analysed using SPSS, version 24. Continuous data were assessed for normality using the Kolmogorov–Smirnov tests and analysed using parametric or non-parametric tests as appropriate. Normal data were expressed as mean ± standard deviation, and non-normally distributed data as median (range). Categorical data were assessed using chi-squared tests. The frequency of the variant was assessed for Hardy–Weinberg equilibrium using chi-squared test. Case–control analyses were performed between patients of matched ethnicity.

Results

Considering the Irish cohort primarily, samples from 724 controls, 523 patients with BC, 136 patients with DTC and 3 patients with dual-primary breast and thyroid cancer were successfully genotyped for rs2910164. The clinical and pathological characteristics of the patient cohort are outlined in Tables 1 and 2. The variant was proven to be in Hardy–Weinberg equilibrium in both case cohorts and in controls.

The frequency of the minor allele was higher in BC cases (0.22) than controls (0.19), but the per allele odds ratio for the C allele did not achieve statistical significance (OR 1.20 (0.98–1.46), $P=0.07$). Neither hetero- nor homozygous genotypes were associated with BC in this patient population (Tables 3 and 4). No significant association was detected between genotype and age at diagnosis of BC ($P=0.197$, Kruskal–Wallis test) or molecular subtype of BC ($P=0.715$, X^2) (Table 5). No association was evident between genotype and T-stage ($P=0.689$, X^2), absolute tumour size ($P=0.327$, Kruskal–Wallis test) or nodal status ($P=0.861$, X^2).

The frequency of the minor allele was significantly higher in DTC cases (0.27) than controls (0.19). The C allele variant was significantly associated with DTC in both heterozygous (OR 1.66 (1.13–2.44), $P=0.009$) and homozygous genotypes (OR 2.24 (1.05–4.78), $P=0.03$) (Tables 3 and 4). When association was analysed by gender, the association remained significant for females. The association also retained significance when histological subtype was considered. When papillary subtypes of thyroid cancer only ($n=110$) were considered, the risk conferred by the CG genotype was 1.55 (1.01–2.38), $P=0.04$; and by the CC genotype, 2.81 (1.3–6.05), $P=0.006$ (Table 5).

We did not identify an association between genotype and age at diagnosis of DTC ($P=0.47$, ANOVA) (Fig. 1). There was no appreciable association between genotype and nodal status ($P=0.728$, X^2) or T-stage ($P=0.079$, X^2).

All three female patients diagnosed with both breast and thyroid primary malignancies were found to be of CG genotype.

One hundred and thirty patients with DTC were recruited from a tertiary centre in the South of France and genotyped successfully for this variant (Tables 6 and 7). This sample demonstrated much greater diversity in terms of ethnic origin – with the majority identifying as 'French Caucasian' ($n=90$, 69%), but significant patients reporting other European ($n=20$, 15%), Asian ($n=7$, 5%) or North African origin ($n=10$, 8%). The frequency of the variant allele in this population was 0.26. There was

Table 1 Irish patient characteristics.

	Breast cancer (*N* (%))	**Thyroid cancer** (*N* (%))	**Controls** (*N* (%))
Total	526*	139* (100)	724 (100)
Gender			
Male	3 (1)	27 (19)	53 (7)
Female	523* (99)	112* (81)	671 (93)
Age at diagnosis (cases) or sampling (controls)			
Median (range)	53 (30–88)	42 (16–84)	70 (60–93)
Mean ± S.D.	55.14 ± 11.11	45.33 ± 15.01	70.72 ± 6.71
Age groups			
15–39	28 (5)	48 (35)	0
40–49	149 (28)	29 (21)	0
50–64	229 (44)	32 (23)	124 (17)
≥65	104 (20)	13 (9)	600 (83)
Unknown	16 (3)	17 (12)	0 (0)

*Including three female patients with breast and thyroid cancer.

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Table 2 Tumour clinico-pathological features.

Breast cancer (*N*=526)		**Thyroid cancer** (*N*=139)	
Histology			
Ductal	397 (75)	Papillary	112 (81)
Lobular	78 (15)	Follicular	27 (19)
Colloid	12 (2)		
Other	19 (4)		
Missing	20 (4)		
Molecular subtype			
Luminal A	344 (65)		
Luminal B	61 (12)		
Her2-overexpressing	28 (5)		
Triple negative	53 (10)		
Unknown	40 (8)		
T-stage			
Is	33 (6)		
1	182 (35)	1	57 (41)
2	216 (41)	2	45 (32)
3	39 (7)	3	25 (18)
4	16 (3)	Unknown	12 (9)
Unknown	37 (7)		
N-stage			
0	250 (48)	0	38
1	130 (25)	1	21
2	62 (12)		
3	27 (5)		
Missing	57 (11)	Not assessed	80

Table 3 Genotypic and allelic frequencies in Irish patients.

Genotype	**Control** (*N*=724)	**DTC** (*N*=139)	**Breast** (*N*=526)
GG	480	74	326
CG	215	55	171
CC	29	10	29
C allele	273	75	229
G allele	1175	203	823
Minor allele frequency	0.19	0.27	0.22
Male individuals only			
Male	**Control** (*n*=53)	**DTC** (*n*=27)	**Breast** (*n*=3)
GG	35 (66)	13 (48)	2 (67)
CG	16 (30)	12 (44)	0
CC	2 (4)	2 (7)	1 (33)
C allele	20	16	2
G allele	86	38	4
Minor allele frequency	0.19	0.30	0.33
Female individuals only			
Female	**Control** (*n*=671)	**DTC** (*n*=112)	**Breast** (*n*=523)
GG	445	61	324
CG	199	43	171
CC	27	8	28
C allele	253	59	227
G allele	1089	165	819
Minor allele frequency	0.19	0.26	0.22

Table 4 Genotypic and allelic odds ratio (Irish patients).

	Odds ratio (95% CI)		
Thyroid cancer			
	Per C allele	CG vs GG	CC vs GG
Overall	1.59 (1.18–2.14) *P*=0.002	1.66 (1.13–2.44) *P*=0.009	2.24 (1.05–4.78) *P*=0.03
Male	1.81 (0.85–3.87) *P*=0.13	2.02 (0.76–5.39) *P*=0.16	2.69 (0.34–21.14) *P*=0.33
Female	1.54 (1.11–2.14) *P*=0.01	1.58 (1.03–2.41) *P*=0.03	2.16 (0.94–4.97) 0.06
Breast cancer			
	Per C allele	CG vs GG	CC vs GG
Overall	1.20 (0.98–1.46) *P*=0.07	1.17 (0.92–1.5), *P*=0.21	1.47 (0.86–2.51) *P*=0.15
Male	2.15 (0.37–12.57) *P*=0.40	0 (n/a)	8.75 (0.54–142.69) *P*=0.77
Female	1.19 (0.98–1.46) *P*=0.09	1.18 (0.92–1.51) *P*=1.19	1.42 (0.82–2.46) *P*=0.20

no significant difference in age at diagnosis (P=0.984), T-stage (0.066) or nodal involvement (P=0.945) between genotypes (Table 8).

Discussion

The role of miRNA-146a and miRNA-146b in inflammation, immune function and epithelial cell homeostasis and their reported roles in inhibition of invasion and metastasis, make them seductive candidates as cancer susceptibility genes. The function of miR-146a, as is true for other microRNAs, appears to be tissue, as well as context specific (51). Different studies have variably categorised miR-146a as tumour suppressor or oncogenic microRNA depending on the tissue of interest (52, 53).

The expression of these miRNAs has been shown to be upregulated in basal-like BC cell lines, a subtype commonly associated with BRCA1 deficiency, compared to luminal subtypes. Binding of these miRNAs to target sites in the 3′UTR of BRCA1 can also downregulate its expression, leading to increased cellular proliferation (54). However, miRNA-146b has also been shown to be upregulated in healthy basal mammary epithelial cells (55). It has also been reported that upregulation of miR-146a/b by BRMS1 leads to inhibition of invasion and metastasis of MDA-MB-231 human breast carcinoma cells (56), by subsequent downregulation of NF-kB through the targets IRAK1 and TRAF6 (57). In other studies, such upregulation was associated with an anti-apoptotic effect in p53-deficient breast tumours (52). The expression of genes involved in the NF-KB pathway and

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Table 5 Genotypic odds ratios depending on molecular subtype of breast cancer or histological subtype of thyroid cancer.

				Genotypic odds ratio (95% CI)			
	GG	**GC**	**CC**	**GC vs CC**		**GG vs CC**	
Molecular subtypes of breast cancer							
Luminal A ($n=342$)	219	106	17	2.02 (1.76–5.39)	$P=0.16$	2.69 (0.34–21.14)	$P=0.33$
Luminal B ($n=61$)	35	22	4	1.4 (0.8–2.45)	$P=0.23$	1.89 (0.63–5.68)	$P=0.25$
Her2-overexpressing ($n=28$)	18	7	3	0.87 (0.36–2.11)	$P=0.75$	1.07 (0.24–2.68)	$P=0.92$
Triple negative ($n=52$)	31	20	2	1.44 (0.8–2.58)	$P=0.22$	2.76 (0.77–9.91)	$P=0.11$
Histological subtypes of thyroid cancer							
Papillary ($n=110$)	59	41	10	1.55 (1.01–2.38)	$P=0.04$	2.81 (1.3–6.05)	$P=0.006$
Follicular ($n=26$)	15	11	0	1.64 (0.74–3.62)	$P=0.2$	n/a	

regulation of apoptosis may also be dependent on pre-miR-146a genotype (53).

Considering rs2910164 in particular, *in vitro* studies have suggested that mature miRNA-146a levels are increased in MCF7 cell lines transfected with pcDNA3.3-miR-146C vs pcDNA3.3-miR-146G, which the authors postulate to be related to increased binding capacity of miR-146a to BRCA1 in the presence of the C-variant (33). However, we, and other authors, have previously shown that circulating miR-146a levels are reduced in the presence of the C allele in patients with BC (58, 59).

miR-146a and miR-146b have been shown to be upregulated and associated with adverse prognostic features, progression and invasion in papillary (60), follicular (61) and anaplastic (62) thyroid cancers. This effect may be mediated through associations with NF-KB (62), ST8SIA4 (61) or RARB (63, 64). A previous study by Jazdzewski and coworkers postulated that different genotypes did not show an association between the homozygous rs2910164 genotype and disease, which they attribute to differential production of mature miRNA in tumours, with heterozygotes producing three alternate isoforms of the miRNA (miR-146a from leading strand, and miR-146a*G and miR-146a*C from passenger strand), with different sets of target genes (24, 65).

Our data support a possible association between the variant allele of rs2910164 and DTC in this patient population. Furthermore, an allele dosage effect was observed, with homozygous genotypes associated with increased odds of disease compared to heterozygotes. In our study, 7% of patients were homozygous for the variant compared to 4% of controls, while in the cohort of the study by Jazdzewski and coworkers the homozygote genotypic frequency was 2.9% in cases compared to 6.6% in controls (24). This study included samples from three ethnically distinct populations (Finland, Poland and USA); with approximately equal representation of cases from each cohort, but over half of the controls were Polish. We have demonstrated that the frequency of the variant allele in French cases is 0.26, which is comparable to that reported in our Irish cases (0.27), and to the MAF in Finnish cases (0.27) (24) and comparatively higher than

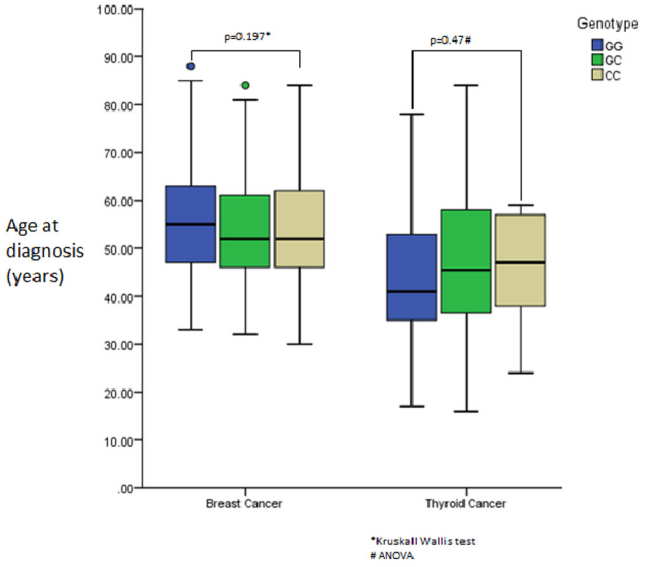

Figure 1
Lack of association between genotype and age at diagnosis.

Table 6 Characteristics of French patients.

Patient characteristic	***N*** (%)
Ethnicity	
French Caucasian	90 (69)
Other European Caucasian	20 (15)
Asian	7 (5)
African	10 (8)
Other	3 (2)
Minor allele frequency	0.26
Mean age at diagnosis ± S.D. (years)	47.71 ± 15.28
Gender	
Male	37 (29)
Female	93 (72)
Histopathological subtype	
Papillary	119 (92)
Follicular	11 (9)

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Table 7 Genotypic frequency in French cohort.

	Patient genotype			
	GG	GC	CC	Significance
N (%)	72 (55)	49 (38)	9 (7)	
Minor allele frequency	0.26			
Mean age at diagnosis ± S.D. (years)	47.85 ± 14.56	47.65 ± 16.27	46.89 ± 17.14	*P* = 0.984, ANOVA
T-stage				
1	40	21	3	0.066, X^2
2	13	12	6	
3	17	12	0	
4	1	0	0	
Unknown	1	4	0	
Nodal status				
0	17	9	2	0.945, X^2
1	22	10	2	
Not assessed	32	27	5	
Unknown	1	3	0	

the MAF reported in the Polish (0.22) (24) or UK (0.24) (32) cohorts, but lower than that reported in American cases (0.30) (24) and considerably lower than that reported in Chinese patients (0.57). A cohort of French controls is required for comparative analyses before any conclusion can be made regarding the significance of this variant in the French population, as there is obvious variability in frequency of the variant that can be demonstrated across different populations. This high MAF in DTC cases merits further investigation in a larger study with controls of matched ethnicity. Variability in frequency of the variant across different populations is exemplified by the different MAF demonstrated in our cohort compared to our nearest geographical neighbours in the United Kingdom, where the MAF in both control and case cohorts was 0.24. The UK study therefore did not support an association

Table 8 Lack of association of genotype with age, T-stage, nodal status.

	Genotype			
	GG	GC	CC	Significance
Breast cancer				
Age	55 (33–88)	52 (32–84)	52 (30–84)	0.197 (Kruskall–Wallis)
T-stage				
Is	24	8	1	0.689, X^2
1	105	65	12	
2	133	72	11	
3	26	10	3	
4	12	3	1	
Unknown	26	10	1	
Tumour size	25 (2–100)	23 (2–116)	25 (2–110)	0.327 (Kruskall–Wallis)
Nodal status				
0	158	77	15	0.861, X^2
1	80	42	8	
2	36	22	4	
3	19	8	0	
Thyroid cancer				
Age	43.89 ± 14.69	47.45 ± 16	46 ± 12.44	0.470 (ANOVA)
T-stage				
1	38	15	4	0.079, X^2
2	21	22	2	
3	10	12	3	
Unknown	5	3	1	
Nodal status				
0	24	12	2	0.728, X^2
1	11	7	2	
Not assessed	39	33	6	

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of the variant with disease. Our criteria for inclusion of participants as controls was much more stringent than in this study, where controls were recruited from the national blood donor service, the 1958 Birth cohort, and from a separate colorectal cancer susceptibility gene discovery project (COloRectal Gene Identification study (CORGI)). Furthermore, although the number of cases included in this study was greater (cases: 748 vs 139; controls: 2857 vs 724); the population in the United Kingdom is 65.6 million (66), almost 14 times greater than that in the Republic of Ireland (approximately 4.7 million (67)). Our sample therefore represents a greater proportion of the national population.

We applied rigorous selection criteria to controls, specifying that they must be aged at least 60 years; the rationale being that patients with a genetic predisposition to cancer are more likely to be affected at younger ages, and older individuals have the lowest residual lifetime possibility of developing cancer. Indeed, the median age at diagnosis of thyroid cancer in our cohort was 45 years (range 16–84). The controls in other studies were young or age-matched unaffected individuals recruited from outpatient clinics or as part of another study (24, 68, 69). In an Italian study, no association between the variant and thyroid cancer was described, but the authors do not describe the ages of the control individuals (43). Young patients have a higher lifetime probability of developing a cancer that may be related to an underlying predisposition.

Previous studies have suggested that gender may influence the strength of the association of rs2910164 with disease (70); potentially because of the regulation of miRNA-146a expression by oestrogen in immune cells (71). In this study, the strength of the association between the variant and thyroid cancer retained significance in female patients, but did not in male patients. A limitation of this study however is the small number of male controls, and this subgroup is underpowered to draw any formal conclusion from the analysis.

As the variant in question is a transversion involving two nucleotides of a Watson–Crick pair; it is important that the orientation of the DNA strand on which the variant is called and the method by which genotyping is performed are considered. Previous meta-analyses have described the multitude of methods by which genotyping of this variant has been performed in different cohorts (72). It is important not just to consider the method, but also the orientation of the primers in the assays utilised and indeed the assembly build on which their design is based. A lack of clarity about this point may have contributed to the apparently discordant results in the literature to date; a confounding factor that has been reported in the investigation of other SNPs (73). The situation in this context is further complicated by the differing frequencies and indeed impact of the variant allele on different populations, especially between East Asian and European Caucasian populations (69, 72; http://exac.broadinstitute.org/variant/5-159912418-C-G).

The technology at our disposal to investigate and identify genetic variation has improved dramatically in recent years. It is therefore likely that the 'low-hanging fruit' – the highly penetrant monogenic cancer predisposition syndromes – have already been identified. The missing heritability of cancer is likely to be attributable to low-penetrance alleles in multiple genes. While individually these polymorphisms may confer small effect sizes, the cumulative risk conferred by inheritance of multiple low-penetrance alleles may possibly approach that of the high-risk monogenic disorders. As we start to develop algorithms to include data from low-penetrance alleles into BC risk estimation (74), we must endeavour to do the same for less common malignancies – particularly those of which the genetic architecture is, as yet, poorly defined.

This study highlights a number of key points. In this cohort, the variant rs2910164 appears to be associated with DTC, but does not have a clear association with BC risk, nor age of disease onset or molecular subtype of BC. The clinical utility of the identification of this variant in a patient sample is, as yet, undetermined, given the numerous potential inflammatory benign and malignant disease processes in which miR-146a has a role; and the differential frequency and influence of this variant across populations. This study is limited by a relatively small number of samples from patients with DTC. However, we believe this sample to be representative of Irish patients with the disease, considering that we have strongly matched for ethnicity in a population of only 4,757,976 (67, 75) with a thyroid cancer incidence of 3.61/100,000 (75).

The possibility of variant allele misalignment between different studies does exist, and a robust meta-analysis, accounting for this, may further elucidate the association between the variant allele at this locus and cancer predisposition.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References

1 Nielsen SM, White MG, Hong S, Aschebrook-Kilfoy B, Kaplan EL, Angelos P, Kulkarni SA, Olopade OI & Grogan RH. The breast-thyroid cancer link: a systematic review and meta-analysis. *Cancer Epidemiology, Biomarkers and Prevention* 2016 **25** 231–238. (doi:10.1158/1055-9965.EPI-15-0833)

2 Ron E, Curtis R, Hoffman DA & Flannery JT. Multiple primary breast and thyroid cancer. *British Journal of Cancer* 1984 **49** 87–92. (doi:10.1038/bjc.1984.13)

3 An JH, Hwangbo Y, Ahn HY, Keam B, Lee KE, Han W, Park DJ, Park IA, Noh DY, Youn YK, *et al*. A possible association between thyroid cancer and breast cancer. *Thyroid* 2015 **25** 1330–1338. (doi:10.1089/thy.2014.0561)

4 Li CI, Rossing MA, Voigt LF & Daling JR. Multiple primary breast and thyroid cancers: role of age at diagnosis and cancer treatments (United States). *Cancer Causes Control* 2000 **11** 805–811. (doi:10.1023/A:1008942616092)

5 Chen AY, Levy L, Goepfert H, Brown BW, Spitz MR & Vassilopoulou-Sellin R. The development of breast carcinoma in women with thyroid carcinoma. *Cancer* 2001 **92** 225–231. (doi:10.1002/1097-0142(20010715)92:2<225::AID-CNCR1313>3.0.CO;2-B)

6 Liaw D, Marsh DJ, Li J, Dahia PL, Wang SI, Zheng Z, Bose S, Call KM, Tsou HC, Peacocke M, *et al*. Germline mutations of the PTEN gene in Cowden disease, an inherited breast and thyroid cancer syndrome. *Nature Genetics* 1997 **16** 64–67. (doi:10.1038/ng0597-64)

7 Chaker L & Visser TJ. Thyroid function: thyroid dysfunction and breast cancer risk—an unfinished story. *Nature Reviews Endocrinology* 2016 **12** 313–314. (doi:10.1038/nrendo.2016.48)

8 Portulano C, Paroder-Belenitsky M & Carrasco N. The Na+/I- symporter (NIS): mechanism and medical impact. *Endocrine Reviews* 2014 **35** 106–149. (doi:10.1210/er.2012-1036)

9 Ryan J, Curran CE, Hennessy E, Newell J, Morris JC, Kerin MJ & Dwyer RM. The Sodium Iodide Symporter (NIS) and potential regulators in normal, benign and malignant human breast tissue. *PLoS ONE* 2011 **6** e16023. (doi:10.1371/journal.pone.0016023)

10 Giani C, Fierabracci P, Bonacci R, Gigliotti A, Campani D, De Negri F, Cecchetti D, Martino E & Pinchera A. Relationship between breast cancer and thyroid disease: relevance of autoimmune thyroid disorders in breast malignancy. *Journal of Clinical Endocrinology and Metabolism* 1996 **81** 990–994. (doi:10.1210/jcem.81.3.8772562)

11 Gogas J, Kouskos E, Tseleni-Balafouta S, Markopoulos C, Revenas K, Gogas G & Kostakis A. Autoimmune thyroid disease in women with breast carcinoma. *European Journal of Surgical Oncology* 2001 **27** 626–630. (doi:10.1053/ejso.2001.1204)

12 Smyth PP, Shering SG, Kilbane MT, Murray MJ, McDermott EW, Smith DF & O'Higgins NJ. Serum thyroid peroxidase autoantibodies, thyroid volume, and outcome in breast carcinoma. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 2711–2716. (doi:10.1210/jcem.83.8.5049)

13 Ling Y, Li Q, Yang H, Wang Y, Tang F, Kang H & Wang Y. Loss of heterozygosity in thyroid hormone receptor beta in invasive breast cancer. *Tumori* 2015 **101** 572–577. (doi:10.5301/tj.5000272)

14 Pal T, Hamel N, Vesprini D, Sanders K, Mitchell M, Quercia N, Ng Cheong N, Murray A, Foulkes W & Narod SA. Double primary cancers of the breast and thyroid in women: molecular analysis and genetic implications. *Familial Cancer* 2001 **1** 17–24. (doi:10.1023/A:1011541424424)

15 Siolek M, Cybulski C, Gąsior-Perczak D, Kowalik A, Kozak-Klonowska B, Kowalska A, Chłopek M, Kluźniak W, Wokołorczyk D, Pałyga I, *et al*. CHEK2 mutations and the risk of papillary thyroid cancer. *International Journal of Cancer* 2015 **137** 548–552. (doi:10.1002/ijc.29426)

16 Pasquinelli AE. MicroRNAs and their targets: recognition, regulation and an emerging reciprocal relationship. *Nature Reviews Genetics* 2012 **13** 271–282. (doi:10.1038/nrg3162)

17 Taganov KD, Boldin MP, Chang KJ & Baltimore D. NF-κB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. *PNAS* 2006 **103** 12481–12486. (doi:10.1073/pnas.0605298103)

18 Hou J, Wang P, Lin L, Liu X, Ma F, An H, Wang Z & Cao X. MicroRNA-146a feedback inhibits RIG-I-dependent Type I IFN production in macrophages by targeting TRAF6, IRAK1, and IRAK2. *Journal of Immunology* 2009 **183** 2150–2158. (doi:10.4049/jimmunol.0900707)

19 Kumaraswamy E, Wendt KL, Augustine LA, Stecklein SR, Sibala EC, Li D, Gunewardena S & Jensen RA. BRCA1 regulation of epidermal growth factor receptor (EGFR) expression in human breast cancer cells involves microRNA-146a and is critical for its tumor suppressor function. *Oncogene* 2015 **34** 4333–4346. (doi:10.1038/onc.2014.363)

20 Garraway LA. A notch for noncoding RNA in melanoma. *New England Journal of Medicine* 2014 **370** 1950–1951. (doi:10.1056/NEJMcibr1402173)

21 Xu B, Huang Y, Niu X, Tao T, Jiang L, Tong N, Chen S, Liu N, Zhu W & Chen M. Hsa-miR-146a-5p modulates androgen-independent prostate cancer cells apoptosis by targeting ROCK1. *Prostate* 2015 **75** 1896–1903. (doi:10.1002/pros.23068)

22 Auton A, Abecasis GR, Altshuler DM, Durbin RM, Abecasis GR, Bentley DR, Chakravarti A, Clark AG, Donnelly P, Eichler EE, *et al*. A global reference for human genetic variation. *Nature* 2015 **526** 68–74. (doi:10.1038/nature15393)

23 Savas S & Liu G. Studying genetic variations in cancer prognosis (and risk): a primer for clinicians. *Oncologist* 2009 **14** 657–666. (doi:10.1634/theoncologist.2009-0042)

24 Jazdzewski K, Murray EL, Franssila K, Jarzab B, Schoenberg DR & de la Chapelle A. Common SNP in pre-miR-146a decreases mature miR expression and predisposes to papillary thyroid carcinoma. *PNAS* 2008 **105** 7269–7274. (doi:10.1073/pnas.0802682105)

25 Chen J, Cao X & Zhang H. MiR-146a rs2910164 polymorphism is associated with hepatocellular carcinoma: a meta-analysis. *International Journal of Clinical and Experimental Medicine* 2015 **8** 15852–15856.

26 Xu Z, Zhang L, Cao H & Bai B. MiR-146a rs2910164 G/C polymorphism and gastric cancer susceptibility: a meta-analysis. *BMC Medical Genetics* 2014 **15** 1–8. (doi:10.1186/s12881-014-0117-2)

27 Bao MH, Xiao Y, Zhang QS, Luo HQ, Luo J, Zhao J, Li GY, Zeng J & Li JM. Meta-analysis of miR-146a polymorphisms association with coronary artery diseases and ischemic stroke. *International Journal of Molecular Sciences* 2015 **16** 14305–14317. (doi:10.3390/ijms160714305)

28 Zhu M, Li D, Jin M & Li M. Association between microRNA polymorphisms and the risk of inflammatory bowel disease. *Molecular Medicine Reports* 2016 **13** 5297–5308. (doi:10.3892/mmr.2016.5157)

29 Li Y, Du C, Wang W, Ma G, Cui L, Zhou H, Tao H, Yao L, Zhao B, Li K, *et al*. Genetic association of MiR-146a with multiple sclerosis susceptibility in the Chinese population. *Cellular Physiology and Biochemistry* 2015 **35** 281–291. (doi:10.1159/000369695)

30 Park R, Lee WJ & Ji JD. Association between the three functional miR-146a single-nucleotide polymorphisms, rs2910164, rs57095329, and rs2431697, and autoimmune disease susceptibility: a meta-analysis. *Autoimmunity* 2016 **49** 451–458.

31 Dong G, Zhang R, Xu J & Guo Y. Association between microRNA polymorphisms and papillary thyroid cancer susceptibility.

http://www.endocrineconnections.org
DOI: 10.1530/EC-17-0196

International Journal of Clinical and Experimental Pathology 2015 **8** 13450–13457.
32 Jones AM, Howarth KM, Martin L, Gorman M, Mihai R, Moss L, Auton A, Lemon C, Mehanna H, Mohan H, *et al*. Thyroid cancer susceptibility polymorphisms: confirmation of loci on chromosomes 9q22 and 14q13, validation of a recessive 8q24 locus and failure to replicate a locus on 5q24. *Journal of Medical Genetics* 2012 **49** 158–163. (doi:10.1136/jmedgenet-2011-100586)
33 Zhang X, Gu Y, Liu X, Yu Y, Shi J, Yu Q, Sun H, Kanu JS, Zhan S & Liu Y. Association of Pre-miR-146a rs2910164 polymorphism with papillary thyroid cancer. *International Journal of Endocrinology* 2015 **2015** article ID 802562. (doi:10.1155/2015/802562)
34 Shen J, Ambrosone CB, DiCioccio RA, Odunsi K, Lele SB & Zhao H. A functional polymorphism in the miR-146a gene and age of familial breast/ovarian cancer diagnosis. *Carcinogenesis* 2008 **29** 1963–1966. (doi:10.1093/carcin/bgn172)
35 Pastrello C, Polesel J, Della Puppa L, Viel A & Maestro R. Association between hsa-mir-146a genotype and tumor age-of-onset in BRCA1/BRCA2-negative familial breast and ovarian cancer patients. *Carcinogenesis* 2010 **31** 2124–2126. (doi:10.1093/carcin/bgq184)
36 Garcia AI, Cox DG, Barjhoux L, Verny-Pierre C, Barnes D; Gemo Study Collaborators, Antoniou AC, Stoppa-Lyonnet D, Sinilnikova OM & Mazoyer S. The rs2910164:G>C SNP in the MIR146A gene is not associated with breast cancer risk in BRCA1 and BRCA2 mutation carriers. *Human Mutation* 2011 **32** 1004–1007. (doi:10.1002/humu.21539)
37 Hu Z, Liang J, Wang Z, Tian T, Zhou X, Chen J, Miao R, Wang Y, Wang X & Shen H. Common genetic variants in pre-microRNAs were associated with increased risk of breast cancer in Chinese women. *Human Mutation* 2009 **30** 79–84. (doi:10.1002/humu.20837)
38 Chen Q-H, Wang Q-B & Zhang B. Ethnicity modifies the association between functional microRNA polymorphisms and breast cancer risk: a HuGE meta-analysis. *Tumor Biology* 2014 **35** 529–543. (doi:10.1007/s13277-013-1074-7)
39 Catucci I, Yang R, Verderio P, Pizzamiglio S, Heesen L, Hemminki K, Sutter C, Wappenschmidt B, Dick M, Arnold N, *et al*. Evaluation of SNPs in miR-146a, miR196a2 and miR-499 as low-penetrance alleles in German and Italian familial breast cancer cases. *Human Mutation* 2010 **31** E1052–E1057. (doi:10.1002/humu.21141)
40 Esteban Cardeñosa E, de Juan Jiménez I, Palanca Suela S, Chirivella González I, Segura Huerta A, Santaballa Beltran A, Casals El Busto M, Barragán González E, Fuster Lluch O, Bermúdez Edo J, *et al*. Low penetrance alleles as risk modifiers in familial and sporadic breast cancer. *Familial Cancer* 2012 **11** 629–636. (doi:10.1007/s10689-012-9563-1)
41 Upadhyaya A, Smith RA, Chacon-Cortes D, Revêchon G, Bellis C, Lea RA, Haupt LM, Chambers SK, Youl PH & Griffiths LR. Association of the microRNA-Single Nucleotide Polymorphism rs2910164 in miR146a with sporadic breast cancer susceptibility: a case control study. *Gene* 2016 **576** 256–260. (doi:10.1016/j.gene.2015.10.019)
42 Alshatwi AA, Shafi G, Hasan TN, Syed NA, Al-Hazzani AA, Alsaif MA & Alsaif AA. Differential expression profile and genetic variants of microRNAs sequences in breast cancer patients. *PLoS ONE* 2012 **7** e30049. (doi:10.1371/journal.pone.0030049)
43 Bansal C, Sharma KL, Misra S, Srivastava AN, Mittal B & Singh US. Common genetic variants in pre-microRNAs and risk of breast cancer in the North Indian population. *ecancermedicalscience* 2014 **8** 473. (doi:10.3332/ecancer.2014.473)
44 Afsharzadeh SM, Ardebili SMM, Seyedi SM, Fathi NK & Mojarrad M. Association between rs11614913, rs3746444, rs2910164 and occurrence of breast cancer in Iranian population. *Meta Gene* 2017 **11** 20–25. (doi:10.1016/j.mgene.2016.11.004)
45 Omrani M, Hashemi M, Eskandari-Nasab E, Hasani SS, Mashhadi MA, Arbabi F & Taheri M. Hsa-mir-499 rs3746444 gene polymorphism is associated with susceptibility to breast cancer in an Iranian population. *Biomarkers in Medicine* 2014 **8** 259–267. (doi:10.2217/bmm.13.118)
46 Meshkat M, Tanha HM, Naeini MM, Ghaedi K, Sanati MH, Meshkat M & Bagheri F. Functional SNP in stem of mir-146a affects Her2 status and breast cancer survival. *Cancer Biomarkers* 2016 **17** 213–222. (doi:10.3233/CBM-160633)
47 Lian H, Wang L & Zhang J. Increased risk of breast cancer associated with CC genotype of has-miR-146a Rs2910164 polymorphism in Europeans. *PLoS ONE* 2012 **7** e31615. (doi:10.1371/journal.pone.0031615)
48 Gao LB, Bai P, Pan XM, Jia J, Li LJ, Liang WB, Tang M, Zhang LS, Wei YG & Zhang L. The association between two polymorphisms in pre-miRNAs and breast cancer risk: a meta-analysis. *Breast Cancer Research and Treatment* 2011 **125** 571–574. (doi:10.1007/s10549-010-0993-x)
49 Zhang H, Zhang Y, Yan W, Wang W, Zhao X, Ma X, Gao X & Zhang S. Association between three functional microRNA polymorphisms (miR-499 rs3746444, miR-196a rs11614913 and miR-146a rs2910164) and breast cancer risk: a meta-analysis. *Oncotarget* 2017 **8** 393–407. (doi:10.18632/oncotarget.13426)
50 Thermo Scientific. Interpretation of nucleic acid 260/280 ratios. In *T123 Technical Bulletin*. Wilmington, DE, USA: Thermo Scientific, 2012.
51 Boufraqech M, Klubo-Gwiezdzinska J & Kebebew E. MicroRNAs in the thyroid. *Best Practice and Research Clinical Endocrinology and Metabolism* 2016 **30** 603–619. (doi:10.1016/j.beem.2016.10.001)
52 Sandhu R, Rein J, D'Arcy M, Herschkowitz JI, Hoadley KA & Troester MA. Overexpression of miR-146a in basal-like breast cancer cells confers enhanced tumorigenic potential in association with altered p53 status. *Carcinogenesis* 2014 **35** 2567–2575. (doi:10.1093/carcin/bgu175)
53 Jazdzewski K & De La Chapelle A. Genomic sequence matters: a SNP in microRNA-146a can turn anti-apoptotic. *Cell Cycle* 2009 **8** 1642–1643.
54 Garcia AI, Buisson M, Bertrand P, Rimokh R, Rouleau E, Lopez BS, Lidereau R, Mikaélian I & Mazoyer S. Down-regulation of BRCA1 expression by miR-146a and miR-146b-5p in triple negative sporadic breast cancers. *EMBO Molecular Medicine* 2011 **3** 279–290. (doi:10.1002/emmm.201100136)
55 Bockmeyer CL, Christgen M, Müller M, Fischer S, Ahrens P, Länger F, Kreipe H & Lehmann U. MicroRNA profiles of healthy basal and luminal mammary epithelial cells are distinct and reflected in different breast cancer subtypes. *Breast Cancer Research and Treatment* 2011 **130** 735–745. (doi:10.1007/s10549-010-1303-3)
56 Hurst DR, Edmonds MD, Scott GK, Benz CC, Vaidya KS & Welch DR. Breast cancer metastasis suppressor 1 up-regulates miR-146, which suppresses breast cancer metastasis. *Cancer Research* 2009 **69** 1279–1283. (doi:10.1158/0008-5472.CAN-08-3559)
57 Bhaumik D, Scott GK, Schokrpur S, Patil CK, Campisi J & Benz CC. Expression of microRNA-146 suppresses NF-kappaB activity with reduction of metastatic potential in breast cancer cells. *Oncogene* 2008 **27** 5643–5647. (doi:10.1038/onc.2008.171)
58 Qi P, Wang L, Zhou B, Yao WJ, Xu S, Zhou Y & Xie ZB. Associations of miRNA polymorphisms and expression levels with breast cancer risk in the Chinese population. *Genetics and Molecular Research* 2015 **14** 6289–6296. (doi:10.4238/2015.June.11.2)
59 Waldron R, McVeigh TP, McVeigh U, Miller N & Kerin MJ. The consequences of a single nucleotide polymorphismin pre-mir-146a and its effect on mature circulating mir-146a levels (abstract). *British Journal of Surgery* 2017 **104** 5–65.
60 Sun M, Fang S, Li W, Li C, Wang L, Wang F & Wang Y. Associations of miR-146a and miR-146b expression and clinical characteristics in papillary thyroid carcinoma. *Cancer Biomarkers* 2015 **15** 33–40. (doi:10.3233/CBM-140431)
61 Ma W, Zhao X, Liang L, Wang G, Li Y, Miao X & Zhao Y. miR-146a and miR-146b promote proliferation, migration and invasion of follicular thyroid carcinoma via inhibition of ST8SIA4. *Oncotarget* 2017 **8** 28028–28041. (doi:10.18632/oncotarget.15885)

http://www.endocrineconnections.org
DOI: 10.1530/EC-17-0196

62 Pacifico F, Crescenzi E, Mellone S, Iannetti A, Porrino N, Liguoro D, Moscato F, Grieco M, Formisano S & Leonardi A. Nuclear factor-κb contributes to anaplastic thyroid carcinomas through up-regulation of miR-146a. *Journal of Clinical Endocrinology and Metabolism* 2010 **95** 1421–1430. (doi:10.1210/jc.2009-1128)
63 Czajka AA, Wójcicka A, Kubiak A, Kotlarek M, Bakuła-Zalewska E, Koperski Ł, Wiechno W & Jażdżewski K. Family of microRNA-146 regulates RARβ in papillary thyroid carcinoma. *PLoS ONE* 2016 **11**. (doi:10.1371/journal.pone.0151968)
64 Jazdzewski K, Boguslawska J, Jendrzejewski J, Liyanarachchi S, Pachucki J, Wardyn KA, Nauman A & de la Chapelle A. Thyroid hormone receptor β (THRB) is a major target gene for microRNAs deregulated in papillary thyroid carcinoma (PTC). *Journal of Clinical Endocrinology and Metabolism* 2011 **96** E546–E553. (doi:10.1210/jc.2010-1594)
65 Jazdzewski K, Liyanarachchi S, Swierniak M, Pachucki J, Ringel MD, Jarzab B & de la Chapelle A. Polymorphic mature microRNAs from passenger strand of pre-miR-146a contribute to thyroid cancer. *PNAS* 2009 **106** 1502–1505. (doi:10.1073/pnas.0812591106)
66 Office for National Statistics. *Overview of the UK population: July 2017*. Newport, South Wales, UK: Office for National Statistics, 2017. (available at: https://www.ons.gov.uk/peoplepopulationandcommunity/populationandmigration/populationestimates/articles/overviewoftheukpopulation/july2017)
67 Central Statistics Office. Census of Population 2016 – Preliminary Results. Cork, Ireland: Central Statisitcs Office, 2016. (available at: http://www.cso.ie/en/releasesandpublications/ep/p-cpr/censusofpopulation2016-preliminaryresults/)
68 Wei W-J, Wang YL, Li DS, Wang Y, Wang XF, Zhu YX, Yang YJ, Wang ZY, Ma YY, Wu Y, *et al*. Association between the rs2910164 polymorphism in pre-Mir-146a sequence and thyroid carcinogenesis. *PLoS ONE* 2013 **8** e56638. (doi:10.1371/journal.pone.0056638)
69 Marino M, Cirello V, Gnarini V, Colombo C, Pignatti E, Casarini L, Diazzi C, Rochira V, Cioni K, Madeo B, *et al*. Are pre-miR-146a and PTTG1 associated with papillary thyroid cancer? *Endocrine Connections* 2013 **2** 178–185. (doi:10.1530/EC-13-0066)
70 Zhou X, Zhu J, Zhang H, Zhou G, Huang Y & Liu R. Is the microRNA-146a (rs2910164) polymorphism associated with rheumatoid arthritis? Association of microRNA-146a (rs2910164) polymorphism and rheumatoid arthritis could depend on gender. *Joint Bone Spine* 2015 **82** 166–171. (doi:10.1016/j.jbspin.2014.12.009)
71 Dai R, Phillips RA, Zhang Y, Khan D, Crasta O & Ahmed SA. Suppression of LPS-induced Interferon-gamma and nitric oxide in splenic lymphocytes by select estrogen-regulated microRNAs: a novel mechanism of immune modulation. *Blood* 2008 **112** 4591–4597. (doi:10.1182/blood-2008-04-152488)
72 Wang AX, Xu B, Tong N, Chen SQ, Yang Y, Zhang XW, Jiang H, Liu N, Liu J, Hu XN, *et al*. Meta-analysis confirms that a common G/C variant in the pre-miR-146a gene contributes to cancer susceptibility and that ethnicity, gender and smoking status are risk factors. *Genetics and Molecular Research* 2012 **11** 3051–3062. (doi:10.4238/2012.August.31.2)
73 Sand PG. A lesson not learned: allele misassignment. *Behavioral and Brain Functions* 2007 **3** 65. (doi:10.1186/1744-9081-3-65)
74 Evans DG, Brentnall A, Byers H, Harkness E, Stavrinos P, Howell A, Newman WG, Cuzick J & FH-risk study Group. The impact of a panel of 18 SNPs on breast cancer risk in women attending a UK familial screening clinic: a case-control study. *Journal of Medical Genetics* 2017 **54** 111–113. (doi:10.1136/jmedgenet-2016-104125)
75 Lennon P, Deedy S, Healy ML, Kinsella J, Timon C & O'Neill JP. Thyroid cancer in Ireland: a 10-year review of the National Cancer Registry. *Endocrine Abstracts* 2015 **37** EP910. (doi:10.1530/endoabs.37.EP910)

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